

## VU Research Portal

### Spontaneous eye blinking, a measure of dopaminergic function in children with acquired immunodeficiency syndrome

Vreugdenhil, H.; Brouwers, P.; Wolters, P.; Bakker, D.J.; Moss, H.

#### **published in**

Archives of Pediatrics and Adolescent Medicine  
1997

#### **DOI (link to publisher)**

[10.1001/archpedi.1997.02170470059011](https://doi.org/10.1001/archpedi.1997.02170470059011)

#### **document version**

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

#### **citation for published version (APA)**

Vreugdenhil, H., Brouwers, P., Wolters, P., Bakker, D. J., & Moss, H. (1997). Spontaneous eye blinking, a measure of dopaminergic function in children with acquired immunodeficiency syndrome. *Archives of Pediatrics and Adolescent Medicine*, 151(10), 1025-1032. <https://doi.org/10.1001/archpedi.1997.02170470059011>

#### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

#### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

#### **E-mail address:**

[vuresearchportal.ub@vu.nl](mailto:vuresearchportal.ub@vu.nl)

# Spontaneous Eye Blinking, a Measure of Dopaminergic Function, in Children With Acquired Immunodeficiency Syndrome

Hestien Vreugdenhil, MA; Pim Brouwers, PhD; Pamela Wolters, PhD; Dirk Bakker, PhD; Howard Moss, PhD

**Objective:** To investigate possible alterations in dopaminergic function in children with acquired immunodeficiency syndrome by evaluating spontaneous eye blink rate, a putative measure of central dopaminergic function.

**Design:** Evaluation of previously videotaped test sessions of a consecutive case series of 50 children (mean age, 5.2 years; range, 2-12 years) with acquired immunodeficiency syndrome.

**Setting:** Government medical research center.

**Results:** Intrarater reliability was high, expected covariation of blink rate with age and concurrent mental activity were confirmed, and obtained rates were similar to published data. Higher blink rates, suggestive of increased dopaminergic function, were associated with more severe cortical atrophy ( $P < .05$ ) and white matter

abnormality ( $P < .05$ ) on computed tomographic brain scans. The presence or severity of basal ganglia calcifications did not seem to influence blink rate. In addition, higher blink rates were associated with higher ratings of depressed affect ( $P < .05$ ) and lower ratings of hyperactive behaviors ( $P < .05$ ) during other test activities.

**Conclusions:** The higher blink rates in human immunodeficiency virus-infected children with more severe cortical abnormalities suggest increased central dopamine activity compared with that in children without cortical computed tomographic brain scan abnormalities. Thus, as a result of structural brain abnormalities, neurotransmitter levels in children with acquired immunodeficiency syndrome may vary and this may be reflected in their socioemotional functioning.

*Arch Pediatr Adolesc Med.* 1997;151:1025-1032

**Editor's Note:** Some readers might wonder why we'd publish this blinking study in the ARCHIVES. Actually we're enthusiastic about mind-brain studies, especially of this caliber.

Catherine D. DeAngelis, MD

From the HIV and AIDS Malignancy Branch, National Cancer Institute, Bethesda (Ms Vreugdenhil and Drs Brouwers, Wolters, and Moss), and Medical Illness Counseling Center, Chevy Chase (Drs Wolters and Moss), Md; and Department of Pediatric Neuropsychology, Free University of Amsterdam, Amsterdam, the Netherlands (Ms Vreugdenhil and Dr Bakker).

**I**N CHILDREN with symptomatic human immunodeficiency virus type 1 (HIV-1) infection, cognitive, motor, socioemotional, and behavioral impairments have been frequently observed.<sup>1-3</sup> These neurodevelopmental deficits have been related to central nervous system (CNS) abnormalities.<sup>4</sup> The predominant cause of the CNS abnormalities seems to be related to the effects of HIV infection of the CNS because opportunistic infections and lymphoma of the CNS are uncommon in children. Evidence of HIV infection has been detected in microglia and macrophages within the CNS, but neurons seem to remain largely uninfected; neurologic dam-

age is postulated to be caused by the release of various neurotoxic viral or host factors.<sup>5,6</sup>

Based on structural, behavioral, and biochemical observations, 1 of the consequences of HIV infection of the CNS could well be the deregulation of the central, nigrostriatal, dopaminergic system. Magnetic resonance imaging, computed tomographic (CT) brain scan studies, and autopsy findings have demonstrated a high incidence of subcortical neuropathologic changes, such as basal ganglia mineralizations (**Figure 1**), and other structural brain abnormalities in children with HIV infection.<sup>7-12</sup> The neurobehavioral profile of children with symptomatic HIV disease is also suggestive of subcortical abnormalities. Among others, psychomotor slowing,<sup>1</sup> relative weakness of expressive compared with receptive language,<sup>13</sup> and lack of facial expressiveness<sup>14</sup>—all have been observed in children with HIV infection. Similar profiles of neurocognitive dysfunctioning in adults with HIV

## SUBJECTS AND METHODS

### SUBJECTS

Patients seen at the Pediatric Branch of the National Cancer Institute, Bethesda, Md, as participants in phase 1 and 2 clinical trials of antiretroviral agents<sup>32,33</sup> were eligible for participation in this study. These protocols were approved by the National Cancer Institute's Institutional Review Board, and written consent was obtained from the parent or legal guardian of each child. As part of comprehensive medical monitoring, patients were evaluated based on CT brain scan findings, age-appropriate neuropsychological test results, which included a videotape procedure,<sup>14</sup> and CD4 count analysis prior to or shortly after (<1 week) starting an antiretroviral treatment protocol. These tests were repeated after 6 months of antiretroviral treatment. Most (66%) of the children previously had been following other antiretroviral treatment protocols with zidovudine or didoxynosine or both, but they were eligible for a new protocol because of disease progression or toxic effects. At the time of the neurobehavioral evaluation, children were afebrile and were not suffering from another infection. Between April 1989 and March 1994, 112 pediatric patients with symptomatic HIV disease were videotaped.

Because the objective of this study was to evaluate the association between HIV-associated CNS abnormalities and behavior, 1 child with Down syndrome, who also had a nystagmus, was excluded from the analysis because of cognitive and neurological abnormalities unrelated to HIV-1 infection.

Children younger than 2 years ( $n=45$ ) were excluded from this study because previous studies had demonstrated that spontaneous blinking in infants and young children is rare and irregular.<sup>26,30</sup> One subject who was 19.1 years old was also excluded because he was 7 years older than the second oldest subject in the sample. The videotapes of 15 subjects were not evaluable according to the criteria described below because the quality of the videotape was poor (the child was positioned badly, or the room was too dark [ $n=13$ ] or because the eyes of the children were not observable long enough as they were too active [ $n=2$ ]). Thus, the videotapes of 50 children were considered evaluable. The mean ( $\pm$ SD) age of these children was  $5.2\pm0.4$  years (**Table**). Most (37 of 50) children acquired HIV-1 by a vertical route of infection. Mean parental education, defined as the average years of education of either the

father or the mother, was 13.4 years. The encephalopathic ( $n=27$ ) and nonencephalopathic group ( $n=23$ ) did not differ in age (mean [ $\pm$ SEM] age,  $5.0\pm0.6$  years vs  $5.3\pm0.7$  years), sex distribution (56% male vs 74% male), route of HIV infection (81% vs 65% vertically acquired infection) or mean ( $\pm$ SEM) level of parental education ( $13.4\pm0.5$  years vs  $13.3\pm0.5$  years).

### PROCEDURES

#### Videotape Evaluation

Children were videotaped for 20 to 30 minutes while engaged in many age-appropriate tasks that were designed to elicit task-oriented, interpersonal-social, sensorimotor, and communicative behaviors, as well as affect.<sup>14</sup> The videotaping procedure has been administered since 1989, at first to all consecutive patients but since July 1, 1992, only to children with evidence of encephalopathy and/or to those younger than 3 years, because of time constraints.

For this study, a videotape was considered evaluable if it contained at least 3 minutes, during which the eyes of the child could be observed and external stimuli, possibly causing blinks, were minimal. The total period for the determination of the blink rate could be obtained by adding several epochs of a minimal length of 1 minute. If more than 1 evaluable videotape was available for a subject, the earliest one was used.

Every videotape was first evaluated to identify, record, and classify epochs that satisfied the above-mentioned criteria. A record was created, which identified the beginning and the end of the epochs; online timing of the epochs was accomplished using the VCR timer. The activity, if it lasted at least 1 minute, was noted. Three distinct mental activities were identified: conversation, listening, and fine-motor performance. If the subject was involved in more than 1 activity during an epoch, that activity was classified as mixed activity.

For each videotape, the record that indicated the beginning and end and the concurrent mental activity for each epoch was copied. The videotapes were then viewed for a second time, and the first copy of the record was used to guide and record the counting of the eye blinks. After evaluating all videotapes, they were viewed for a third time and a blank copy of the record was used to record the second counting of the eye blinks.

have been interpreted as reflecting subcortical dementia<sup>15</sup> and basal ganglia disease.<sup>16</sup>

Biochemical evidence for decreased activity of the central dopaminergic system has come from adult patients, especially those with acquired immunodeficiency syndrome (AIDS) dementia complex. Some studies<sup>17,18</sup> have reported lower concentrations of dopamine or its metabolite homovanillic acid in the cerebrospinal fluid of patients with AIDS. Furthermore, lower levels were related to lower CD4 lymphocyte counts and to the presence of neurologic and cognitive or motor deficits.

A recent report<sup>19</sup> has shown improvement in motor function with levodopa therapy in a small sample of HIV-infected children with extrapyramidal syndromes, which may suggest abnormal dopaminergic function. Central dopaminergic function, however, has not been studied directly or indirectly. A putative and noninvasive method to evaluate central dopaminergic activity that involves both D<sub>1</sub> and D<sub>2</sub> dopamine receptors is through the spontaneous eye blink rate.<sup>20</sup> This approach has been validated in neurochemical studies with humans<sup>21</sup> and across different species<sup>22,23</sup> and in studies of patients with known dopaminergic

## Neuropsychological Evaluation

The general level of cognitive functioning was assessed with age-appropriate standardized tests. A general index of mental abilities (GIMA) was defined as the Full-Scale Intelligence Quotient of the Wechsler Intelligence Scale for Children-Revised,<sup>34</sup> which was administered to patients aged 6 through 16 years (n=22); the General Cognitive Index of the McCarthy Scales of Children's Abilities,<sup>35</sup> which was administered to children aged 30 months through 6 years (n=14); and the Mental Developmental Index of the Bayley Scales of Infant Development,<sup>36</sup> which was administered to infants aged 2 to 30 months (n=14). For children and infants who scored below the deviation scale cutoff on the Bayley and McCarthy Scales (n=4), ratio IQs were calculated as described elsewhere,<sup>32</sup> based on the age equivalent for the obtained raw score on the test and the child's chronological age. All GIMA scores less than 40 were set to 39 for equivalence and computational purposes.<sup>37</sup>

## Socioemotional Behavior

The psychologist administering the cognitive tests also rated the child's socioemotional behavior during the psychometric evaluation using the National Institutes of Health Q-sort behavior rating procedure<sup>38</sup> (n=41). This is a forced-choice rating procedure resulting in item scores between 1 and 7. Scaled scores are calculated based on an average equal-weight computation of items loading on derived factors from a factor analysis. The Q-sort procedure generates 4 scales, which reflect the following behaviors: depression, hyperactivity or attention deficit, autism, and low frustration and/or irritability threshold.

## Neuroimaging Evaluations

For 46 of the 50 children, a CT brain scan was available; it was obtained within 4 months of the videotape procedure. These scans were rated using a previously described, highly reliable semiquantitative technique.<sup>8</sup> Briefly, 2 neurologists, blind to the clinical status of the patients, independently rated the CT scans for the presence and severity of ventricular dilatation, subarachnoid enlargement, white matter abnormalities, intracerebral calcifications, and possible other lesions on 100-mm analog scales. In addition, an overall rating was given, which served as a composite for all observed abnormal findings. The average of the raters' scores was used.

## Immunological Evaluation

The CD4% was determined as an indicator of stage of HIV disease. Because of rapid physiological changes in normal CD4 levels in the first 4 years of life, a formula was used to transform the data to age-adjusted standard (z) scores.<sup>39,40</sup> We used CD4% rather than absolute CD4 values because its variability is significantly smaller<sup>41</sup> and the age-adjustment is more uniform,<sup>39</sup> which makes it a more reliable measure and better suited for our current study. The CD4 counts within a week from the date of the videotapes were available for 48 of the subjects.

## HIV-ENCEPHALOPATHY CLASSIFICATION

Children were classified as encephalopathic or nonencephalopathic, based on the following nonmutually exclusive criteria, which have been used consistently in our program<sup>32,33</sup> (because of the lack of a generally accepted classification for HIV-associated encephalopathy of childhood,<sup>42,43</sup> we recognize that these criteria may differ in other institutions): (1) evidence of neurocognitive deterioration (loss of skills or milestones) in functioning (n=9), (2) general level of functioning more than 2 SDs below norm (GIMA, <70; n=19), and (3) general level of functioning between 1 and 2 SDs below the norm and evidence of moderate to severe CNS abnormality on CT brain scan (score, >49 on the analog rating for overall severity of abnormality<sup>8</sup>; n=6). Patients who did not fulfill any of these 3 criteria were classified as nonencephalopathic (n=23).

## DATA ANALYSIS

Blink rates (in bpm) were calculated for each of the classified cognitive activities (conversation, listening, and fine motor) and an overall blink rate for each videotape. A square root transformation was standardly applied, because the data were positively skewed, which normalized the scores. We back-transformed the data when presenting them in tables and graphs.

Intraclass reliabilities<sup>44</sup> between the first and second counting were computed. As the intrarater reliabilities were appropriate (see the "Results" section), the average of the 2 counts was used in all subsequent analyses.

As noted before, because the blink rate is age dependent, analysis of covariance and partial correlation were used for adjusting for age effects. Additionally, data were analyzed with the *t* test analysis of variance, Fisher exact test, and Pearson correlation. A minimal 2-tailed  $\alpha$  level of .05 was used in all analyses.

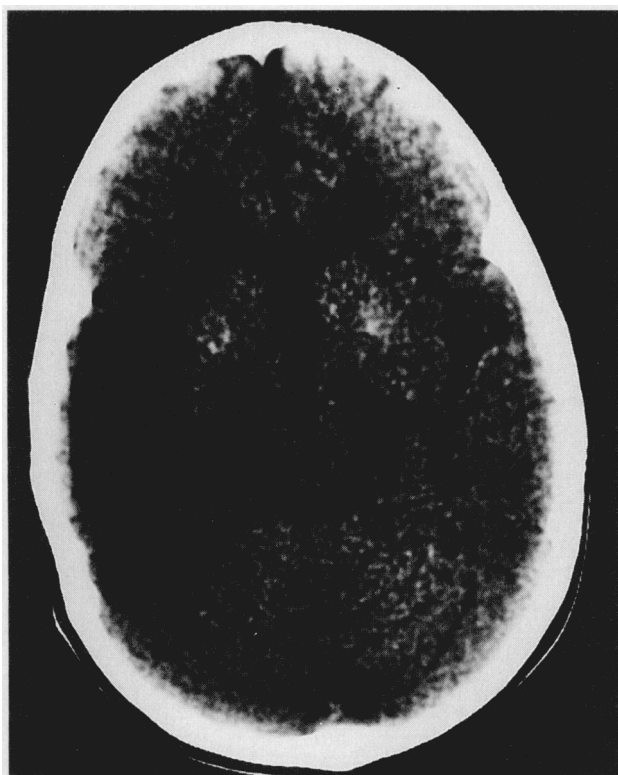
dysfunction such as Parkinson disease<sup>24-26</sup> and schizophrenia.<sup>20,27</sup> Lower blink rates are associated with decreased activity of the central dopaminergic system.

Cortical control of the spontaneous eye blink rate is evidenced by its modulation with concurrent mental activity. The blink rate is significantly reduced during visually demanding tasks<sup>26,28</sup> or during tasks requiring concentration and mental activity,<sup>29</sup> and it is significantly increased during casual conversation<sup>28</sup> compared with blink rates during rest conditions. Blink rates remain constant when subjects are involved in a single activity.<sup>26</sup> Developmentally, blink rates increase significantly from an irregular and very

low rate of 0.7 blinks per minute (bpm) in infants to a rate of about 17 bpm by age 20 years, when it remains relatively constant.<sup>30</sup> The effect of concurrent mental activity on the blink rate is similar for children and adults.<sup>31</sup>

In this study, the spontaneous eye blink rates in children with symptomatic HIV disease, which were obtained from videotaped behavior samples, were evaluated. Our purpose was to ascertain whether lower age-adjusted blink rates were associated with the following: presence of encephalopathy, greater severity of brain imaging abnormalities, and more advanced status of HIV disease, as reflected by lower age-adjusted CD4 percentage (CD4%) values.





**Figure 1.** Computed tomographic brain scan of a 4.5-year-old patient with vertically acquired human immunodeficiency virus infection and classified as encephalopathic. Severity of the bilateral calcifications in the basal ganglia was rated in the mild-to-moderate range. Cortical atrophy, reflected by both enlargement of the subarachnoid spaces and dilatation of the ventricles, was rated in the moderate-to-severe range.

## RESULTS

### GENERAL SAMPLE DESCRIPTION

#### Psychometric Data

The general level of cognitive functioning for this sample of pediatric HIV-infected patients (mean [ $\pm$ SEM] GIMA,  $77.7 \pm 3.5$ ) was more than 1 SD below the published norm (100). The mean [ $\pm$ SEM] GIMA of the encephalopathic group ( $60.0 \pm 2.8$ ) was significantly lower ( $P < .001$ ) than that of the nonencephalopathic children ( $98.5 \pm 3.5$ ).

In the socioemotional domain, encephalopathic children exhibited significantly more depressive (mean [ $\pm$ SEM] score,  $3.6 \pm 0.3$  vs  $2.8 \pm 0.2$ ;  $P < .05$ ) and autistic (mean [ $\pm$ SEM] score,  $2.6 \pm 0.2$  vs  $1.5 \pm 0.1$ ;  $P < .001$ ) behaviors compared with the nonencephalopathic children on the Q-sort rating procedure. Differences on the hyperactivity or attention deficit ( $P > .50$ ) and irritability or low frustration threshold ( $P > .20$ ) scales were not significant. The mean levels on these 4 scales were comparable to earlier reported levels for these HIV subgroups.<sup>38</sup>

The GIMA score correlated significantly with the autism rating ( $R = -0.60$ ;  $P < .001$ ), but with none of the other 3 scales of the socioemotional behavior rating.

### Characteristics of the Children With Symptomatic Human Immunodeficiency Virus Infection

Characteristics	All Children	Group	
		Encephalopathic	Nonencephalopathic
Sex, M/F ratio	32/18	15/12	17/6
Route of infection	37/13	22/5	15/8
vertical/ transfusion ratio			
Age, y*	$5.15 \pm 3.0$	$5.04 \pm 2.9$	$5.29 \pm 3.2$
Parental education, y	13.4	13.4	13.3
GIMA*†	$77.7 \pm 24.8$	$60.0 \pm 14.7$	$98.5 \pm 16.7$
Calcifications present, No.	13	12	1
CD4%, z scores*‡	$-3.27 \pm 1.5$	$-4.03 \pm 0.9$	$-2.37 \pm 1.6$

\*Data are mean  $\pm$  SD.

†GIMA indicates general index of mental abilities (defined in the "Neuropsychological Evaluation" subsection of the "Procedures" section in "Subjects and Methods").

‡Age-corrected z scores for CD4 lymphocyte subset percentages (CD4%).

### Neuroimaging (CT Scan) Findings

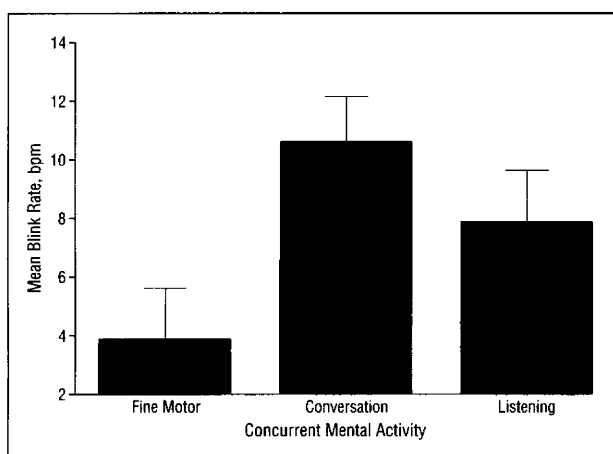
Almost all children (98%) in this sample were rated as having at least 1 abnormal finding on the CT brain scan. For more than half the subjects, the CNS abnormalities were rated as minimal or mild (32% and 24%, respectively). For 35% of the children, the CNS abnormalities were rated as moderate; for 9% of the children, as severe. The most frequently seen lesion was cortical atrophy (ventricular dilatation or subarachnoid enlargement or both<sup>8</sup>). Thirteen subjects (28%) had calcifications in the basal ganglia or in the frontal white matter or in both sites. As has been noted previously,<sup>8</sup> these lesions were more common ( $P < .01$ ) in vertically infected (12 [32%] of 37) compared with transfusion-infected children (1 [8%] of 13). The overall severity rating of the CT brain scan abnormalities in the encephalopathic group (mean [ $\pm$ SEM],  $56.5 \pm 13.4$ ) was significantly higher ( $P < .001$ ) than in the nonencephalopathic group (mean [ $\pm$ SEM],  $22.6 \pm 3.6$ ).

Computed tomographic brain scan abnormalities, particularly cortical atrophy, were related with a lower GIMA ( $R = -0.59$ ;  $P < .001$ ) and higher abnormality ratings on the depression ( $R = 0.51$ ;  $P < .005$ ) and the autism ( $R = 0.51$ ;  $P < .005$ ) scales of the Q-sort procedure.

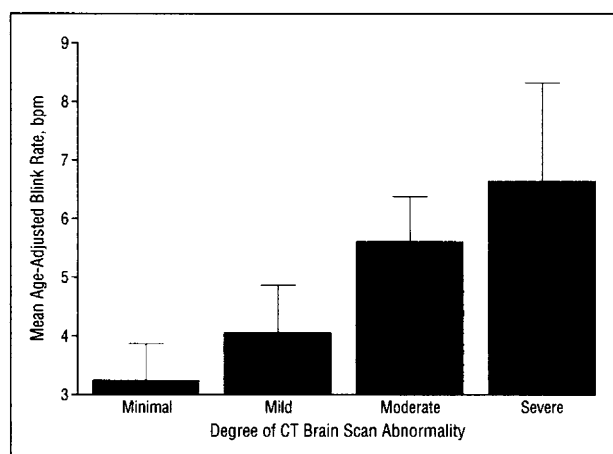
### Immunologic Findings

The overall age-adjusted level of CD4% was significantly below the reported level<sup>39</sup> of non-HIV-infected children ( $z = -3.27 \pm 0.22$ ). As expected, the level of immune depletion for the encephalopathic children ( $z = -4.03 \pm 0.17$ ) was significantly greater ( $P < .001$ ) than for the nonencephalopathic children ( $z = -2.37 \pm 0.35$ ).

Similarly, age-adjusted CD4% correlated with overall CT brain scan abnormality ( $R = -0.54$ ;  $P < .001$ ), particularly cortical atrophy ( $R = -0.53$ ), and with the level of cognitive functioning ( $R = 0.45$ ;  $P < .005$ ).



**Figure 2.** Relation between mean blink rates (in blinks per minute [bpm]) and concurrent mental activity in children with symptomatic human immunodeficiency virus disease. Significantly higher blink rates were seen during conversation ( $P < .01$ ) and listening ( $P < .05$ ) compared with those during a fine-motor task. The difference between conversation and listening was not significant ( $P > .40$ ).



**Figure 3.** Comparison of mean blink rates (in blinks per minute [bpm]) in children with symptomatic human immunodeficiency virus disease, classified as having a minimal (analog rating, 0-24;  $n = 15$ ), mild (analog rating, 25-49;  $n = 11$ ), moderate (analog rating, 50-74;  $n = 16$ ), or severe (analog rating, 75-100;  $n = 4$ ) degree of computed tomographic (CT) brain scan abnormality ( $R = 0.37$ ;  $P < .05$ ).

## RELIABILITY AND VALIDITY

### Intrarater Reliability

Intrarater reliability was highly significant for both the overall blink rate and its square root transformation ( $R = 0.99$ ). Similarly, significant reliability coefficients were found for the blink rate during conversation ( $n = 15$ ;  $R = 0.97$ ), listening ( $n = 13$ ;  $R = 0.98$ ), and fine-motor performance ( $n = 31$ ;  $R = 0.997$ ).

### Effect of Concurrent Mental Activity on Blink Rate

There were significant differences among the mean blink rates during conversation, listening, and fine-motor performance ( $F = 14.39$ ;  $P < .001$ ) (**Figure 2**). These statistics should be considered with some caution, because complete data about all 3 conditions were only available for 8 subjects. Post hoc pairwise comparisons demonstrated that blink rates were significantly lower during fine-motor tasks compared with those during conversation ( $n = 12$ ;  $F = 21.64$ ;  $P < .001$ ) and listening ( $n = 11$ ;  $F = 7.73$ ;  $P < .02$ ). The difference between blink rates during listening and conversation ( $n = 9$ ;  $F = 0.71$ ;  $P > .50$ ) was not significant.

The overall blink rate correlated significantly with the blink rates for the 3 activities: fine-motor performance ( $R = 0.67$ ;  $P < .001$ ), conversation ( $R = 0.88$ ;  $P < .001$ ), and listening ( $R = 0.59$ ;  $P < .04$ ). Thus, the use of an overall blink rate was justified as a composite measure.

## CORRELATIONS WITH BLINK RATE

### CT Brain Scan Variables

Greater severity of overall CT brain scan abnormalities were significantly associated with higher age-adjusted blink rates (partial correlation coefficient, 0.37;  $P < .05$ ) (**Figure 3**). When evaluating the different CT brain scan abnormalities that make up the overall CT rating, severity of cortical atrophy ( $R = 0.37$ ;  $P < .05$ ), and of white matter abnormalities

( $R = 0.35$ ;  $P < .05$ ), but not severity of calcifications ( $R = 0.15$ ;  $P > .30$ ), were significantly related to higher blink rates.

In addition, blink rates of children with calcifications ( $n = 13$ ) and without calcifications ( $n = 33$ ) were compared. Because children with calcifications had significantly ( $P < .02$ ) higher severity of cortical atrophy (mean [ $\pm$ SEM] rating,  $50.2 \pm 5.8$ ) compared with that of children without calcifications (mean [ $\pm$ SEM] rating,  $29.9 \pm 4.4$ ), analysis of covariance was used to adjust for cortical atrophy and age. The adjusted mean ( $\pm$ SEM) blink rates of the 2 groups were almost identical ( $4.50 \pm 0.53$  vs  $4.49 \pm 0.81$ ).

### Disease and Demographic Variables

Relations between blink rate and sex or route of infection were not significant. There was a nonsignificant trend for higher blink rates to be associated with lower age-adjusted CD4% values ( $R = -0.25$ ;  $P = .10$ ).

### Psychological Variables

Blink rate was not significantly related to the GIMA ( $R = -0.22$ ;  $P = .14$ ). Significant relations, however, were found with the behavior rating measures for the depression ( $R = 0.32$ ;  $P < .05$ ) and the hyperactivity or attention deficit ( $R = -0.32$ ;  $P < .05$ ) scales.

### SUBGROUP DIFFERENCES IN BLINK RATES: ENCEPHALOPATHY

The difference in age-adjusted blink rates between encephalopathic and nonencephalopathic children ( $4.85 \pm 0.60$  bpm vs  $3.85 \pm 0.62$  bpm) was not statistically significant ( $P = .25$ ).

### COMMENT

Spontaneous eye blink frequency was used in this study as a putative measure to evaluate central dopaminergic

activity in children with symptomatic HIV infection. Abnormalities of the dopaminergic system were hypothesized because a subset of children with HIV infection have a profile of neurobehavioral deficits suggestive of subcortical abnormalities, and approximately one third of the children with vertically acquired infection have neuropathologic changes in the basal ganglia.<sup>8</sup> Moreover, decreased cerebrospinal fluid levels of dopamine or its metabolite homovanillic acid have been reported in adult patients with AIDS and were related to the severity of neurologic deficits.<sup>17,18</sup> In addition, improvements in motor functioning with levodopa therapy have been reported in HIV-infected children with extrapyramidal syndromes.<sup>19</sup> Therefore, decreased blink rates could be expected in children with more advanced HIV-associated neurological disease, especially in patients with calcifications in the basal ganglia.

**O**UR RESULTS, however, do not seem to support these hypotheses. Higher blink rates, which have been associated with increased central dopaminergic activity, were related to greater severity of CT brain scan abnormalities, particularly with more severe cortical atrophy and white matter abnormality. Blink rates of children with basal ganglia calcifications were no different from those of children without calcifications. There was a nonsignificant trend for blink rates to be higher, rather than lower, in children with HIV-associated encephalopathy compared with nonencephalopathic patients.

The method used in the current study to measure the spontaneous blink rate seems to be valid and reliable. The intrarater intraclass reliabilities were acceptable. Moreover, our data replicated a number of common characteristics of the blink rate seen in normal children and adults. Concurrent mental activity significantly modulated spontaneous eye blink rates in a way similar to that in normal children and adults.<sup>28,31</sup> First, blink rates were significantly elevated during conversation and listening compared with those during fine-motor tasks. Second, the mean blink rates in the current study were comparable to previously published rates in normal children<sup>30</sup> and showed a comparable increase with age (for children between 1-5 years of age, 3.4 bpm in normal children vs  $3.8 \pm 0.4$  bpm in our study; for children between 5-10 years of age, 6.1 bpm in normal children vs  $5.6 \pm 0.9$  bpm in our study).

Further validation of our procedure is provided by the relation that we obtained between blink rates and depression. Blink rate correlated significantly with the depression ratings from the Q-sort procedure,<sup>38</sup> particularly with such items as "seems sad" and "doesn't smile or laugh." Increased blink rates also have been reported for adult patients with depression<sup>45</sup> and depressive affective disorder.<sup>46</sup>

In general, brain imaging abnormalities and encephalopathy in pediatric patients with AIDS have been related to cognitive dysfunctioning and aberrant socio-emotional behavior patterns. An exception were hyperactive behaviors that have been associated with the ab-

sence of encephalopathy,<sup>38</sup> less severe cortical atrophy,<sup>47</sup> and more intact immune function.<sup>47</sup> In non-HIV-infected children, hyperactivity has been related to lower levels of dopamine.<sup>48,49</sup> A recent study<sup>50</sup> also reported lower blink rates for children with attention deficit hyperactivity disorder who were not receiving stimulant medication compared with those for normal children. These findings support the data from the current study that higher ratings on the hyperactivity or attention deficit scale of the Q-sort behavior rating procedure (particularly on items such as "is excitable and impulsive") were related to lower blink rates. However, HIV-infected children with hyperactive behaviors may only be a small subgroup. In a recent retrospective study of 116 children with symptomatic HIV disease (H.M., unpublished data, January 8, 1997), we found that the prevalence of significant hyperactive behaviors, defined as scoring more than 2 SDs above the norm on the Conners' Rating Scales,<sup>51</sup> was only 10%.

Spontaneous blink rates are partially controlled by a neural system with inhibitory modulation from the cerebellum and occipital cortex.<sup>52</sup> Increased blink rates may signify the disinhibition of this neural system.<sup>53</sup> After lesions of the cerebellum or cerebellectomy in children<sup>53</sup> and animals,<sup>54</sup> increased spontaneous blink rates have been demonstrated. Furthermore, blinks are suppressed during visual and electrical activity in the visual cortex.<sup>55</sup> Moreover, after lesions in the prefrontal cortex, an increase in subcortical dopamine level has been demonstrated in rats.<sup>56</sup> The structural cortical abnormalities in our patients, therefore, may cause a down-regulation of the cortical inhibitory system<sup>52</sup> and an up-regulation of the dopaminergic system of the basal ganglia, resulting in an increase in spontaneous blinking. Studies in schizophrenic patients, who are hypothesized to have chronically high dopaminergic function,<sup>57</sup> tend to support this interpretation. Schizophrenic patients with enlarged ventricles seemed to respond less to treatment with neuroleptic drugs (dopamine antagonists) than did comparable patients without enlarged ventricles.<sup>58</sup> Additionally, the increased blink rates in schizophrenic patients with enlarged ventricles were not decreased by neuroleptic therapy, whereas the blink rate in those patients with normal ventricles did decrease with similar doses of neuroleptic drugs.<sup>27</sup>

There is not much insight yet how basal ganglia calcifications in pediatric HIV disease affect the functioning of the basal ganglia. A recent functional brain imaging study in 8 vertically infected children reported basal ganglia hypermetabolism in children with basal ganglia calcification.<sup>59</sup> Hypermetabolism in the basal ganglia has also been reported for adults, in whom basal ganglia calcifications were absent, in the early, but not late, stages of AIDS dementia complex.<sup>60</sup> Similarly, our findings do not suggest a reduction in dopaminergic activity, as measured by blink rate, in children with basal ganglia calcifications compared with children without calcifications.

Despite significant correlations of cortical atrophy with blink rate and with general level of cognitive abilities, the correlation between blink rate and general level of cognitive abilities did not reach statistical signifi-



cance. This suggests that the blink rate may reflect other aspects of CNS function affected by HIV disease than those measured by tests of general cognitive function. Previous studies<sup>2,38</sup> have demonstrated that socioemotional behaviors also may be compromised by HIV-1-associated CNS disease in children. The significant correlations of blink rate with hyperactivity or attention deficit and depression ratings suggest that blink rates may be more reflective of the socioemotional domain.

The findings of our study, suggesting increased dopaminergic function in children with more severe CNS abnormalities, seem at variance with the adult HIV studies<sup>17-18</sup> and the pediatric AIDS treatment study<sup>19</sup> that suggested decreased dopaminergic function. One reason for these differences could be that the stage of (CNS) HIV infection itself may have a direct influence on neurotransmitter levels. Animal studies<sup>61</sup> have shown that CNS infections may raise central dopamine levels, particularly in the acute phase. Furthermore, *in vitro* studies have shown that increased production of noradrenaline, an associated neurotransmitter, is related to higher levels of HIV-1 viral products, such as gp-120, in a human neural brain cell line.<sup>62</sup>

It is possible that, in our study, the more acute HIV infection, including the CNS, may have led to comparatively higher dopamine levels than are those in the patients in the other studies. Most children in our study either had not been treated with antiviral drugs before or showed progressive disease while receiving an antiviral protocol at the time of enrollment to the study.<sup>32-33</sup> We have previously shown that HIV-1 provirus is detected more often at autopsy in both the cerebrum and cerebellum of children than of adults with AIDS and at higher viral burden, as shown with the use of polymerase chain reaction.<sup>63,64</sup>

Further studies directly measuring dopamine or its metabolite homovanillic acid level in the cerebrospinal fluid of children with symptomatic HIV disease are necessary to support the findings and conclusions of this study. However, unbiased collection of cerebrospinal fluid samples from infants and children is difficult. Administering lumbar punctures and spinal taps to children in general requires a clinical justification, and that very clinical event may influence the levels of the compound under study.

In summary, this study demonstrated increased spontaneous blink rates in HIV-positive children, with more severe degrees of CT brain scan abnormality, particularly cortical atrophy and white matter lesions. Because spontaneous blink rates are a putative measure of central dopaminergic activity, these findings suggest increased dopaminergic activity in this subgroup of children with symptomatic HIV disease.

Accepted for publication June 25, 1997.

A preliminary portion of this study was presented at the 19th Annual Mid-Year Meeting of the International Neuropsychological Society, June 20, 1996, Veldhoven, the Netherlands. The abstract was published as Vreugdenhil H, Moss H, Wolters P, Bakker D, Brouwers P. Evaluation of spontaneous eye blinking as a putative measure of central dopaminergic function in children with symptomatic HIV-1 infection. *J Int Neuropsychol Soc.* 1996;2:190. Abstract.

We express our appreciation for the time and effort given by the children and their caregivers who participated in this research. We also thank Charles DeCarli, MD, and Lucy Civitello, MD, for their evaluation of the CT brain scans, and Philip Pizzo, MD, Eugene Tassone, Renee Smith, MA, Debra El-Amin, MA, Nancy Heilman, PhD, and Gayl Selkin-Gutman, MA, for their assistance during the various phases of this project.

Reprints: Pim Brouwers, PhD, Pediatric Neuropsychology Group, National Cancer Institute, National Institutes of Health Bldg 10, Room 13N240, Bethesda, MD 20892-1928 (e-mail: pb56u@nih.gov).

## REFERENCES

1. Aylward EH, Butz AM, Hutton N, Joyner ML, Vogelhut JW. Cognitive and motor development in infants at risk for human immunodeficiency virus. *AJDC.* 1992; 146:218-222.
2. Belman AL, Brouwers P, Moss H. HIV-1 and the central nervous system. In: Kaufman DM, Solomon GE, Pfeffer CR, eds. *Child and Adolescent Neurology for Psychiatrists.* Baltimore, Md: Williams & Wilkins; 1992:238-253.
3. Epstein LG, Sharer LR, Oleske JM, et al. Neurologic manifestations of human immunodeficiency virus infection in children. *Pediatrics.* 1986;78:678-687.
4. Brouwers P, DeCarli C, Civitello LA, Moss H, Wolters P, Pizzo P. Correlation between computed tomographic brain scan abnormalities and neuropsychological function in children with symptomatic human immunodeficiency virus disease. *Arch Neurol.* 1995;52:39-44.
5. Epstein LG, Gendelman HE. Human immunodeficiency virus type 1 infection of the nervous system: pathogenic mechanisms. *Ann Neurol.* 1993;33:429-436.
6. Lipton SA. HIV-related neuronal injury: potential therapeutic intervention with calcium channel antagonists and NMDA antagonists. *Mol Neurobiol.* 1994;8:181-196.
7. Chamberlain MC, Nichols SL, Chase CH. Pediatric AIDS: comparative cranial MRI and CT scans. *Pediatr Neurol.* 1991;7:357-362.
8. DeCarli C, Civitello LA, Brouwers P, Pizzo PA. The prevalence of computed axial tomographic abnormalities of the cerebrum in 100 consecutive children symptomatic with the human immunodeficiency virus. *Ann Neurol.* 1993;34:198-205.
9. Kauffman WM, Sivit CJ, Fitz CR, Rakusan TA, Herzog K, Chandra RS. CT and MR evaluation of intracranial involvement in pediatric HIV infection: a clinical-imaging correlation. *Am J Neuroradiol.* 1992;13:949-957.
10. Kure K, Llena JF, Lyman WD, et al. Human immunodeficiency virus-1 infection of the nervous system. *Hum Pathol.* 1991;22:700-710.
11. Price DB, Inglesse CM, Jacobs J, et al. Pediatric AIDS: neuroradiologic and neurodevelopmental findings. *Pediatr Radiol.* 1988;18:445-448.
12. Wiley CA, Belman AL, Dickson DW, Rubinstein A, Nelson JA. Human immunodeficiency virus within the brains of children with AIDS. *Clin Neuropathol.* 1990; 9:1-6.
13. Wolters PL, Brouwers P, Moss HA, Pizzo PA. Differential receptive and expressive language functioning of children with symptomatic HIV disease and relation to CT scan brain abnormalities. *Pediatrics.* 1995;95:112-119.
14. Moss HA, Wolters PL, Brouwers P, Hendricks ML, Pizzo PA. Impairment of expressive behavior in pediatric HIV-positive patients with evidence of CNS disease. *J Pediatr Psychol.* 1996;21:379-400.
15. Brouwers P, Mohr E, Hildebrand K, et al. A novel approach to the determination and characterization of HIV dementia. *Can J Neurol Sci.* 1996;23:104-109.
16. Martin A. HIV, cognition, and the basal ganglia. In: Grant I, Martin A, eds. *Neuropsychology of HIV Infection.* New York, NY: Oxford University Press; 1994: 234-259.
17. Berger JR, Kumar M, Kumar A, Fernandez JB, Levin B. Cerebrospinal fluid dopamine in HIV-1 infection. *AIDS.* 1994;8:67-71.
18. Larsson M, Hagberg L, Forsman A, Norkrans G. Cerebrospinal fluid catecholamine metabolites in HIV-infected patients. *J Neurosci Res.* 1991;28:406-409.
19. Mintz M, Tardieu M, Hoyt L, McSherry G, Mendelson J, Oleske J. Levodopa therapy improves motor function in HIV-infected children with extrapyramidal syndromes. *Neurology.* 1996;47:1583-1585.
20. Karson CN. Spontaneous eye-blink rates and dopaminergic systems. *Brain.* 1983; 106:643-653.
21. Blin O, Masson G, Azulay JP, Fondaraj J, Serratic G. Apomorphine-induced blinking and yawning in healthy volunteers. *Br J Clin Pharmacol.* 1990;30:769-773.



22. Karson CN, Staub RA, Kleinman JE, Wyatt RJ. Drug effect on blink rates in rhesus monkeys: preliminary studies. *Biol Psychiatry*. 1981;16:249-254.
23. Elsworth JD, Lawrence MS, Roth RH, et al. D<sub>1</sub> and D<sub>2</sub> dopamine receptors independently regulate spontaneous blink rate in vervet monkey. *J Pharmacol Exp Ther*. 1991;259:595-600.
24. Hall A. The origin and purposes of blinking. *Br J Ophthalmol*. 1945;89:507-516.
25. Karson CN, Burns S, LeWitt PA, Foster NL, Newman RP. Blink rates and disorders of movements. *Neurology*. 1984;34:677-678.
26. Ponder E, Kennedy WP. On the act of blinking. *Quart J Exp Physiol*. 1927;18:89-119.
27. Kleinman JE, Karson CN, Weinberger DR, Freed WJ, Berman KF, Wyatt RJ. Eye-blinking and cerebral ventricle size in chronic schizophrenic patients. *Am J Psychiatry*. 1984;141:1430-1432.
28. Karson CN, Berman KF, Donnelly EF, Mendelson WB, Kleinman JE, Wyatt RJ. Speaking, thinking, and blinking. *Psychiatr Res*. 1981;5:243-246.
29. Telford CW, Thompson N. Some factors influencing voluntary and reflex eyelid responses. *J Exp Psychol*. 1933;16:524-539.
30. Zemetkin AJ, Stevens JR, Pittman R. Ontogeny of spontaneous blinking and of distribution of the reflex blink. *Ann Neurol*. 1979;5:453-457.
31. Caplan R, Guthrie D. Blink rate in childhood schizophrenia spectrum disorder. *Biol Psychiatry*. 1994;35:228-234.
32. Brouwers P, Moss H, Wolters P, et al. Effect of continuous infusion zidovudine therapy on neuropsychologic functioning in children with symptomatic human immunodeficiency virus infection. *J Pediatr*. 1990;117:980-985.
33. Butler KM, Husson RN, Balis FM, et al. Dideoxyinosine (ddi) in symptomatic HIV-infected children: a phase I-II study. *N Engl J Med*. 1991;324:137-144.
34. Wechsler D. *Manual for the Wechsler Intelligence Scale for Children*. New York, NY: Psychological Corp; 1974.
35. McCarthy D. *Scales of Children's Abilities*. New York, NY: Psychological Corp; 1972.
36. Bayley N. *Manual for the Bayley Scales of Infant Development*. San Antonio, Tex: Psychological Corp; 1969.
37. Brouwers P, Belman A, Epstein L. Central nervous system involvement: manifestations and evaluation. In: Pizzo PA, Wilfert CM, eds. *Pediatric AIDS: The Challenge of HIV Infection in Infants, Children, and Adolescents*. Baltimore, Md: Williams & Wilkins; 1991:318-335.
38. Moss HA, Brouwers P, Wolters PL, Wiener L, Hersh SP, Pizzo PA. The development of a Q-sort behavior rating procedure for pediatric HIV patients. *J Pediatr Psychol*. 1994;19:27-46.
39. European Collaborative Study. Age-related standards for T lymphocyte subsets based on uninfected children born to human immunodeficiency virus-infected women. *Pediatr Infect Dis J*. 1992;11:1018-1026.
40. Brouwers P, Tudor-Williams G, DeCarli C, et al. Relation between stage of disease and neurobehavioral measures in children with symptomatic HIV disease. *AIDS*. 1995;9:713-720.
41. Raszka WV, Meyer GA, Waecker NJ, et al. Variability of serial absolute and percent CD4<sup>+</sup> lymphocyte counts in healthy children born to human immunodeficiency virus 1-infected parents. *Pediatr Infect Dis J*. 1994;13:70-72.
42. Janssen RS, Cornblath DR, Epstein LG, McArthur J, Price RW. Human immunodeficiency virus (HIV) infection and the nervous system: report from the American Academy of Neurology AIDS Task Force. *Neurology*. 1989;39:119-122.
43. Belman AL. Acquired immunodeficiency syndrome and the child's central nervous system. *Pediatr Clin North Am*. 1992;39:691-714.
44. Bartko JJ, Carpenter WT. On the methods and theory of reliability. *J Nerv Ment Dis*. 1976;163:307-317.
45. Mackintosh JH, Kumar R, Kitamura T. Blink rate in psychiatric illness. *Br J Psychiatry*. 1983;143:55-57.
46. Depue RA, Iacono WG, Muir R, Arbisi P. Effect of phototherapy on spontaneous eye blink rate in subjects with seasonal affective disorder. *Am J Psychiatry*. 1988;145:1457-1459.
47. Brouwers P, van der Vlugt H, Moss H, Wolters P, Pizzo P. White matter changes on CT brain scan are associated with neurobehavioral dysfunction in children with symptomatic HIV disease. *Child Neuropsychol*. 1995;1:93-105.
48. Levy F. The dopamine theory of attention deficit hyperactivity disorder (ADHD). *Austr N Z J Psychiatry*. 1991;25:277-283.
49. McCracken JT. A two-part model of stimulant action on attention-deficit hyperactivity disorder in children. *J Neuropsychiatry Clin Neurosci*. 1991;3:201-209.
50. Caplan R, Guthrie D, Komo S. Blink rate in children with attention-deficit hyperactivity disorder. *Biol Psychiatry*. 1996;39:1032-1038.
51. Conners CK. *Conners' Rating Scales, Manual*. North Tonawanda, NY: Multi-Health Systems Inc; 1990.
52. Karson CN. Blinking. *Bull Soc Belg Ophtalmol*. 1989;237:443-457.
53. Karson CN, Dykman RA, Paige SR. Blink rates in schizophrenia. *Schizophr Bull*. 1990;16:345-354.
54. Freed WJ, Karson CN, Kleinman JE, Wyatt RJ. Increased spontaneous eye-blinks in cerebellectomized rats. *Biol Psychiatry*. 1981;16:789-792.
55. Volkman FC, Riggs LA, Moore RK. Eyeblinks and visual suppression. *Science*. 1980;207:900-902.
56. Pycocck CJ, Kerwin RW, Carter CJ. Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats. *Nature*. 1980;286:74-77.
57. Meltzer HY. Biochemical studies in schizophrenia. In: Bellak L, ed. *Disorders of the Schizophrenic Syndrome*. New York, NY: Basic Books; 1979:45-135.
58. Weinberger DR, Bigelow LB, Kleinman JE. Cerebral ventricular enlargement in chronic schizophrenia: association with poor response to treatment. *Arch Gen Psychiatry*. 1980;37:619-623.
59. Depas G, Chiron C, Tardieu M, et al. Functional brain imaging in HIV-1 infected children born to seropositive mothers. *J Nucl Med*. 1995;36:2169-2174.
60. Rottenberg DA, Moeller JR, Strocher SC, et al. The metabolic pathology of the AIDS dementia complex. *Ann Neurol*. 1987;22:700-706.
61. Lycke E, Roos BE. The monoamine metabolism in viral encephalitides of the mouse. *Brain Res*. 1972;44:603-613.
62. Pittaluga A, Pattarini R, Severi P, Raiteri M. Human brain N-methyl-D-aspartate receptors regulating noradrenaline release are positively modulated by HIV-1 coat protein gp 120. *AIDS*. 1996;10:463-468.
63. Sei S, Kleiner DE, Kopp JB, et al. Quantitative analysis of viral burden in tissues from adults and children with symptomatic human immunodeficiency virus type 1 infection assessed by polymerase chain reaction. *J Infect Dis*. 1994;170:325-333.
64. Sei S, Saito K, Stewart SK, et al. Increased HIV-1 DNA content and quinolinic acid concentration in brain tissues obtained from AIDS patients with HIV encephalopathy. *J Infect Dis*. 1995;172:638-647.